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Synthesis and study of antioxidant activity of hydrazone and thiosemicarbazidebased on N-morpholinoacetic acid hydrazide

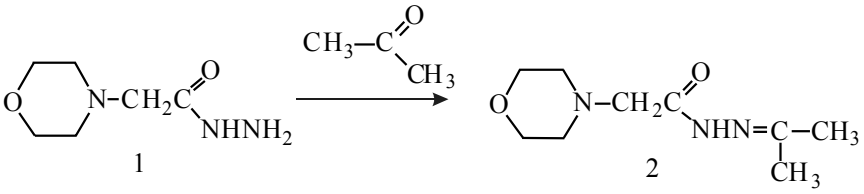
Data on the synthesis and study of the effect of 2-morpholino-N-(propane-2-ylidene) acetohydrazide and 2-(2-morpholinoacetyl)-N-phenylhydrazinocarbothioamide hydrochloride on the electroreduction of oxygen (EV O_2) in various concentrations were presented. The structures of synthesized compounds were studied by IR and ^{1}H NMR spectroscopy methods. Cathode voltammetry on a mercury-film electrode was used as a method for estimating the antioxidant activity of samples. In this approach, the determination of antioxidant activity reflected the number of active forms of oxygen neutralized by the antioxidant in a certain time. The change in the current of the O_2 OV in its absolute value indicated that the samples under study reacted with oxygen and its active radicals in the test solution. The degree of change in the current of the O_2 OV was an indicator of the activity of the sample under study. A similar voltammogram was obtained for the substance under study. A decrease in the cathodic current of E2 O_2 is observed, which indicates that they exhibit antioxidant activity with respect to this process. In addition, there was a shift in the potential of the cathode current of the O_2O_2 to the positive potential region. All of the above implies the existence of an EU mechanism (electrochemical — chemical stage), which includes the subsequent chemical reaction of the interaction of antioxidants with active oxygen radicals. It is shown that the aqueous solution of the studied morpholine derivatives exhibits antioxidant activity.

Keywords: 2-morpholino-N-(propane-2-ylidene)acetohydrozide, 2-(2-morpholinoacetyl)-N-phenylhydrazinocarbothioamide^{1}H NMR spectrum, IR spectrum, antioxidative activity, cathodic voltammetry method, voltammograms, thiosemicarbazide.

It is known that a metabolic transformation of substances in the human body generates free toxic oxygen radicals [1, 2]. They are formed during reactions of lipid peroxidation, metabolism of various drugs or due to impact of external factors (the action of ultraviolet radiation, ionizing radiation). Once they are formed in the body, radicals interact with cell structures, ultimately resulting to cell membranes shock, thus accompanying the development of pathological processes of many diseases. Normally the regulation of the products of activated oxygen metabolites and free radicals in human organs and tissues is performed by vitamins, pigments, hormones, and enzymes. Despite the high efficiency of the antioxidant system, it is not always able to protect the body against the development of oxidative stress. In this regard, one of the priorities is to produce preparation having antioxidant properties with the purpose of use in the prevention and treatment of diseases. In this perspective the synthesis based on hydrazine containing morpholine derivatives are of considerable interest since morpholine-containing fragment is a structural element of numerous natural and synthetic bioactive substances. It should be noted that practically all morpholine derivatives have a broad spectrum of biological activity such as an anti-pyretic, anti-inflammatory, antimicrobial, anti-bacterial, anti-tuberculosis, and anti-cancer one. In order to continue the targeted research to find new opportunities on obtaining new bioactive derivatives with antioxidant effect, N-morpholylaceticacid hydrazide was selected as a synthon.

This work provides the synthesis and study of antioxidative activity of 2-morpholino-N-(propane-2-ylidene)acetohydrazide and 2-(2-morpholinoacetyl)-N-phenylhydrazinocarbothioamide in regard to oxygen radicals by cathodic voltammetry method.

It is known [3, 4], that hydrazones are widely used in synthetic chemistry due to their simple production method and diverse biological activity. The most common method of hydrazones synthesis is the condensation of hydrazines from carbonyl compounds [5]. Thus, we synthesized 2-morpholino-N-(propane-2-ylidene)acetohydrazide (2) obtained with condensing of N-morpholinylaceticacidhydrazide (1) with acetone. Product 2 appeared to be a well crystallizing white substance that is soluble in many organic solvents with the yield of 79 %.



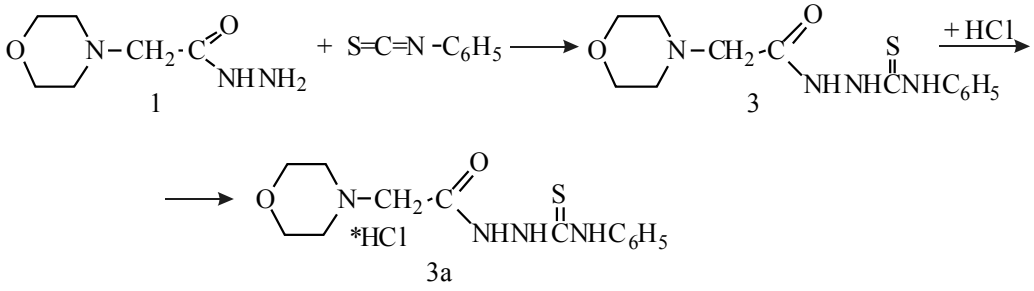
1 2

The IR spectrum of compound 2 have troughs at 3185–3255 cm^{-1} that correspond to stretching vibrations of N–H group, and troughs at 1675–1690 cm^{-1} correspond to stretching vibrations of C=O groups of hydrazone.

In the ^1H NMR spectrum of 2-morpholino-N-(propane-2-ylidene)acetohydrazide (2) signals of methylene protons of morpholine fragment are represented as two triplets centered in 2.52 and 3.39 ppm. Methylene protons of NCH_2-fragments appear at the 3.52 ppm as the narrow singlet. There are two intensive singlets of two methyl groups at 1.75 ppm and 1.95 ppm in the strong field. Singlet in region of 6.21 ppm belongs to N–H proton.

It should be noted that the sulfur-containing functional group in some medicines can be the main element determining the bioactivity, while in others it can just have a certain influence on the pharmacological effect. The biological effect of sulfur-containing compounds first used as drugs appeared due to their antibacterial properties. In this regard, the particular interest is in thiosemicarbazides and their derivatives.

In continuation of research on the synthesis of new biologically active compounds, particularly thiosemicarbazide 3,N-morpholinylacetic acid hydrazide (1), we studied reaction of its condensation with phenylisothiocyanate in alcohol medium at equimolar ratios of the reactants. The reaction proceeds in quiet mild conditions for the synthesis in good yield of the desired product (90 %). Synthesized thiosemicarbazide derivative 3 is a white crystalline powder, soluble in polar organic solvents. In order to obtain water-soluble form of 2-(2-morpholinoacetyl)-N-phenylhydrazinocarbothioamide (3) hydrochloride 3a was derived.



1 3 3a

There are absorption bands in the IR spectrum of compound 3 at 1140–1240 cm^{-1}, which is characteristic for the NH–CS group of the thiosemicarbazide fragment and amide group C(O)NH at 1690–1675 cm^{-1} as well as NH group at 3390–3360 cm^{-1}.

Protons of the phenyl ring are presented in the ^1H NMR spectrum of N-morpholinylacetic acid N–phenylthiosemicarbazide (3) along with the methylene protons signals of the morpholine fragment (2.45 ppm and 3.61 ppm) and NCH_2 fragment (3.07 ppm) in a weak field 7.15–7.34 ppm. Amide and thioamide NH protons are appearing in weak fields in the form of three singlets in the region of 9.85 ppm, 9.80 ppm and 7.98 ppm.

It is known that free radicals are an important part of the pathogenesis of many diseases [1]. Therefore, using voltammetric analyzer at various concentrations in aqueous solution isan important chapter in studying pharmacological activity of 2-morpholino-N-(propane-2-ylidene)acetohydrazide (2) and hydrochloride of 2-(2-methylmorpholinoacetyl)-N-phenylhydrazinocarbothioamide (3a).

The activity of the samples was determined by using cathodic voltammetry, particularly the electrochemical reduction of oxygen (O_2 EV). Model reaction of O_2 EV proceeds by a mechanism similar to oxygen reduction in body cells and tissues:

$$O_2 + e^- \leftrightarrow O_2^{\cdot -}$$
$$O_2^{\cdot -} + H^+ \leftrightarrow HO_2^{\cdot}$$
$$HO_2^{\cdot} + H^+ + e^- \leftrightarrow H_2O_2$$
$$H_2O_2 + 2H^+ + 2e^- \leftrightarrow 2H_2O$$

In this case, the first wave of O_2 EV (reduction of oxygen to hydrogen peroxide) with formation of active oxygen species: O_2, HO^{2-} is under consideration. It is assumed that substances react with oxygen and its active radicals on the indicative electrode surface, which is reflected in changes of O_2 EV cathode current.

The activity of the studied substance with respect to O_2 EV was determined by the following procedure: O_2 EV voltammograms were recorded in the absence of studied substance (background curve). In the absence of extraneous peaks background solution was considered as pure. Then, studied substance with known concentration was added in a cell (for 10 mL supporting electrolyte volume) and cathodic O_2 EV voltammograms were obtained in the same conditions. The measurements were repeated at least 3 times over the defined period of time and value of O_2 EV current limit was estimated each time.

O_2 EV Slope by its absolute value indicates that the studied samples react with oxygen and its active radicals in the test solution. Degree of O_2 EV current change is an indicator of the activity of the test sample.

For the same type of the substance voltammogram is obtained and cathode current decrease is observed in the O_2 EV, which indicates the exertion of antioxidant activity relating to this process. In addition, there was a shift in potential cathode current of O_2 EV to the positive potentials area. All aforesaid requires a ES mechanism (electrochemical — chemical steps), which comprises the subsequent chemical reaction of the antioxidants with active oxygen radicals.

Antioxidant activity of 2-morpholino-N-(propane-2-ylidene)acetohydrazide(2) and 2-(2-morpholinoacetyl)-N-phenylhydrazino carbothioamide (3a) with different concentrations are shown in the Table.

T a b l e

Antioxidant activity of the compounds (2 and 3a) with different concentrations

Substance concentration, g/mL	Average value mole/L	
	Compound 2	Compound 3a
0.00001	0.1432	0.1512
0.0001	0.2355	0.3039
0.001	0.4153	0.4624

The obtained data (Table) demonstrates that hydrochloride of 2-(2-methylmorpholinoacetyl)-N-phenylhydrozinocarbothioamide (3a) expresses the highest antioxidant activity at the maximal analyzed concentration. In the same time moderate growth of antioxidant activity of 2-morpholino-N-(propane-2-ylidene) acetohydrazide (2) is observed as well. It is shown that even at the lowest concentration studied substance reacts with oxygen and its active forms, deactivating them in the solution. Antioxidant activity in this case can be considered as a useful feature that extends the potential range of uses of the researched compounds.

Thus, hydrazone and thiosemicarbazide synthesis was performed on the base of N-morpholinyl acetic acid hydrazide and the influence on the electrochemical reduction of oxygen (O_2EV) at different concentrations was investigated by cathodic voltammetry using the mercury coated electrode. In this approach, determination of antioxidant activity reflects the amount of reactive oxygen species antioxidant neutralizes over time. It is demonstrated that an aqueous solution of researched compounds exhibits antioxidant activity against oxygen radicals.

Experimental

1-H NMR spectrum was recorded on the Bruker 400 spectrometer at 400 MHz in DMSO-d_6 solution relative to the internal standard TMS. Melting point was determined on a Boetius instrument. Progress of the reaction and purity of the obtained compound was monitored by thin layer chromatography on the Silufol UV-254plates in the system of isopropanol – benzene – 25 % ammonia solution 10:5:2. The plates were displayed with iodine vapors. Hydrazide of N-morpholinoacetic acid was prepared as described in [6].

2-Morpholino-N-(propane-2-ylidene)acetohydrazide (2).10 mL of acetone is added to the 1.59 g (0.01 mol) of N-hydrazide of morpholine acetic acid. The mixture is heated for 10 minutes to dissolve hydrazide. The mixture is kept at room temperature until fine-grained sediment precipitates. Completion of the reaction is detected with TLC. Re-crystallization from petroleum ether gives 1.57 g (79 %) of a white powdery substance with m. p. 95–97 ºC. ^{1}H-NMR (DMSO-d_6), δ, ppm.: 2.52 t (4H, N(CH_2)$_2$, J_{HH} 4.66), 3.39 s (2H, NCH_2), 3.56 t (4H, O(CH_2)$_2$, J_{HH}4.65), 6.21 s (1H, N<u>H</u>N), 1.75 s (3H, C<u>H</u>$_3$),1.95 s (3H, C<u>H</u>$_3$).

N-Phenylthiosemicarbazide of N-morpholinylacetic acid (3). 1.59 g (0.01 mole) of N-morpholinylacetic acidhydrazide is dissolved in 5 mL of ethanol, then 1.48 g (0.011 mole) of phenylthiocyanate is added by drops. Mixture is mixed during 30 min at 50–60 ºC. Completion of the reaction is detected with TLC. Solu-

tion is cooled, precipitated fine-grained sediment is filtered and rinsed with small amount of petroleum ether. After re-crystallization from benzene 2.64 g (90 %) of compound 3 with m.p. 147–150 ºC was obtained. ^{1}H NMR spectrum (500 MHz, DMSO-d_6, δ, ppm, *J*/Hz): 2.45 t (4H, $N(CH_2)_2$, J = 4.3), 3.07 b.r.s (2H, N-CH_2), 3.61 t (4H, $O(CH_2)_2$, J_{HH} 4.6), 7.15–7.34 m (5H, Ar), 9.85 s [1H, NH(C=S)], 7.98 s (1H, NH), 9.80 s [1H, NH(C=O)].

Hydrochloride of N-morpholinylacetic acid N-phenylthiosemicarbazide (3a). Saturated with gaseous HCl benzene solution is added to the obtained product 3 dissolved in benzene. Presipitated fine-grained white powder is filtered and rinsed with absolute benzene several times.

Reagent preparation. Stock solutions of researched substances at a concentration of 0.1 g/ml are prepared as follows: using analytical balance 0.5 g sample was weighed and dissolved in 5 ml of distilled water. Further, 0.01 g/ml and 0.001 g/ml solutions are prepared with followed dilutions with distilled water in 10 ml vials. Aliquots of 0.1 ml volume were taken for studies obtaining appropriate working concentrations of test solutions presented in the table.

The voltammetric analysis method used in this work is expressive, with high sensitivity (10^{-10}) and broad analytical capabilities. The developed technique is unique, reproducible, and does not require long-term sample preparation.

Experiment methodology of antioxidant activity determination included obtaining and analysis of the voltammograms of cathodic O_2 EV using voltammetric analyzer connected to the computer [7]. Direct current mode of cathodic voltammetry was used, potential sweep speed W = 40 mV/s, the working range of potentialis in range from 0.0 to 1V, solution stirring time is 20 seconds, settling time is 10 seconds. Electrochemical cell was a glass beaker containing an indicator of mercury coated electrode, silver chloride reference electrode and a silver chloride auxiliary electrode immersed into supporting electrolyte solution. Phosphate buffer solution was chosen as supporting electrolyte in a volume of 10 mL and with pH 6.86, which is close to the physiological value.

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N-морфолинилсірке қышқыл гидразиді негізінде гидразондар мен тиосемикарбазидтердің синтезі мен тотығуға қарсы белсенділікті анықтау

Мақалада 2-морфолино-N-(пропан-2-илиден)ацетогидразидпен 2-(2-морфолиноацетил)-N- фенилгидразинокарботиоамид гидрохлоридінің синтездері бойынша мәліметтер мен әртүрлі концентрацияда (ЭВ O_2) оттектің электрлі қайта қалпына келтіру үрдісіне әсері келтірілген. Синтезделген қосылыстардың құрылымы ИҚ- мен ЯМР ^{1}Н-спектроскопия әдістерімен зерттелген. Тотығуға қарсы белсенділіктің бағалау тәсілі ретінде сынапты-қабыршықты электродта катодты вольтамперометрия қолданылған. Тотығуға қарсы белсенділікті анықтау тәсілінде белгілі бір уақытта тотығуға қарсы бейтараптандырылған оттегінің белсенді түрлерінің мөлшері келтірілген. ЭВ O_2 тоғының өзінің

абсолютті мәнінің өзгеруі зерттелінген қосылыстардың белгілі бір ерітіндіде белсенді радикалдармен және оттегімен әрекеттескенін күәландырады. ЭВ O_2 тоғының өзгеру дәрежесі зерттелінетін заттың белсенділігінің көрсеткіші болып табылады. Зерттелінетін зат үшін біртипті вольтамперограмма алынды, ЭВ O_2 катодты тоғының азаюы байқалған. Бұл осы үрдіс бойынша тотығуға қарсы белсенділіктің пайда болуын дәлелдейді. Сонымен қатар ЭВ O_2 катодты тоғының потенциалының оң аймаққа ығысуы белгілі. Жоғарыда айтылғандардың барлығы ЕК (электрохимиялық — химиялық кезеңдер) тотығуға қарсы белсенді оттекті радикалдармен химиялық әрекеттесу реакциясына түсетін механизмнің барын болжайды. Зерттелінген морфолин туындыларының сулы ерітінділері тотығуға қарсы белсенділікке ие екендігі көрсетілді.

Кілт сөздер: 2-морфолино-N-(пропан-2-илиден)ацетогидразид, 2-(2-морфолиноцетил)-N-фенилгидразинокарботиоамид, ^{1}H ЯМР-спектрі, ИҚ-спектрі, тотығуға қарсы белсенділік, катодтық вольтамперометрия әдісі, вольтамперограмма, тиосемикарбазид.

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Синтез и изучение антиоксидантной активности гидразонов и тиосемикарбазидов на основе гидразида N-морфолиноуксусной кислоты

В статье приведены данные по синтезу и изучению влияния 2-морфолино-N-(пропан-2-илиден)-ацетогидразида и гидрохлорида 2-(2-морфолиноацетил)-N-фенилгидразинокарботиоамида на процесс электровосстановления кислорода (ЭВ O_2) в различных концентрациях. Исследовано строение синтезированных соединений методами ИК- и ЯМР ^{1}H-спектроскопии. В качестве метода оценки антиоксидантной активности образцов применена катодная вольтамперометрия на ртутно-пленочном электроде. В данном подходе определение антиоксидантной активности отражало количество активных форм кислорода нейтрализованных антиоксидантом за определенное время. Изменение тока ЭВ O_2 по своему абсолютному значению свидетельствует о том, что исследуемые образцы реагируют с кислородом и его активными радикалами в исследуемом растворе. Степень изменения тока ЭВ O_2 являлась показателем активности исследуемого образца. Для исследуемого вещества получена однотипная вольтамперограмма, наблюдалось уменьшение катодного тока ЭВ O_2, что свидетельствует о проявлении ими антиоксидантной активности по отношению к данному процессу. Кроме того, наблюдался сдвиг потенциала катодного тока ЭВ O_2 в положительную область потенциалов. Все сказанное выше предполагает наличие механизма ЕС (электрохимическая — химическая стадии), который включает последующую химическую реакцию взаимодействия антиоксидантов с активными кислородными радикалами. Показано, что водный раствор изученных производных морфолина проявляет антиоксидантную активность.

Ключевые слова: 2-морфолино-N-(пропан-2-илиден)ацетогидразид, 2-(2-морфолиноацетил)-N-фенилгидразинокарботиоамид, ^{1}H ЯМР-спектр, ИК-спектр, антиокислительная активность, метод катодной вольтамперометрии, вольтамперограмма, тиосемикарбазид.

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